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Dehydrated strawberries for probiotic delivery: Influence of dehydration and probiotic incorporation methods

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ABSTRACT

In this study, dehydrated strawberries have been proposed as probiotic carriers. Strawberries were cut into halves, incorporated with probiotic *Bacillus coagulans* BC4 by two alternative methods (impregnation and alginate coating) and submitted to two alternative drying methods (freeze drying - FD - and oven drying - OD). Six treatments were carried out, namely: FD and OD (no probiotic), I-FD, I-OD, C-FD, and C-OD (I- and C- meaning impregnation and coating respectively). While the probiotic incorporation method affected a few properties of the resulting products (mainly the probiotic viability on processing), the drying methods resulted in remarkable differences. The freeze-dried strawberry halves presented higher retention of chemical (ascorbic acid and anthocyanin contents) and physical properties (shape, color, and firmness) as well as a better acceptance and higher probiotic viability, resulting in higher probiotic release into the small intestine. The I-FD treatment resulted in the highest probiotic viability after processing and through a 6-month storage (near 8 log cfu.g^{-1}).

1. Introduction

The global market for probiotics is expected to reach USD 76.7 billion by 2027, motivated by the growing consumer awareness regarding their health benefits, including their expected positive effects on the immune responses to covid-19 (Meticulous Research, 2020). The global sales for probiotic foods has far outweighed that of probiotic supplements (USD 41 billion *versus* USD 3.8 billion, in 2015) (Feldman, Lowery, Zambetti, & Madit, 2018). Dairy foods are still the most common probiotic food products, but there has been an increasing demand for non-dairy products, which meet the needs of people with dietary restrictions to dairy foods (including vegans and vegetarians as well as people with lactose intolerance or allergy reactions to milk proteins), and a variety of non-dairy matrices has demonstrated potential as probiotic carriers, as reviewed elsewhere (Min, Bunt, Mason, & Hussain, 2019).

Most studies with probiotic food products use bacteria from the Lactobacillaceae family or *Bifidobacterium* genus (Betoret et al., 2019; Dias et al., 2018; Ribeiro et al., 2020; Vivek, Mishra, & Pradhan, 2020), most of which do not form spores, which makes them sensitive to harsh processing conditions. Spore-forming probiotic bacteria, on the other hand, have increased resistance to environmental stresses. Those are usually from the *Bacillus* genus (Salvetti et al., 2016), including *Bacillus coagulans*, which produces coagulin, a bacteriocin with a broad antimicrobial activity (Kapse, Engineer, Gowdaman, Wagh, & Dhakephalkar, 2019). *B. coagulans* BC4 has exhibited a high stability on storage and digestion of a dried date paste (Marcial-Coba, Pjaca, Andersen, Knøchel, & Nielsen, 2019). When compared to a *Lactobacillus acidophilus* control, *B. coagulans* MTCC 5856 was about five times more resistant to simulated digestion conditions (Shinde et al., 2019).

A number of fruit products has been proposed for probiotic delivery, including fruit juices (Dias et al., 2018; Olivares, Soto, Caballero, & Altamirano, 2019) and fruit powders (Alves et al., 2017; Paim, Costa, Walter, & Tonon, 2016; Vivek et al., 2020). Dehydrated fruits have also been presented as probiotic carriers, the probiotics being usually incorporated by impregnation from a probiotic suspension, including simple impregnation at atmospheric pressure (Akman, Uysal, Ozkaya, Tornuk, & Durak, 2019; S.; Rodrigues, Silva, Mulet, Cárcel, & Fernandes,

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2018; Valerio et al., 2020), vacuum impregnation (Cui et al., 2018; Noorbakhsh, Yaghmaee, & Durance, 2013; Valerio et al., 2020), or osmotic dehydration-assisted impregnation (Emser, Barbosa, Teixeira, & Morais, 2017; Rascón et al., 2018). Probiotic-carrier coatings, on the other hand, have been more commonly applied to minimally processed (Bambace, Alvarez, & Moreira, 2019; Khodaei & Hamidi-Esfahani, 2019; F. J.; Rodrigues, Cedran, & Garcia, 2018) rather than dehydrated fruits. While the impregnation approach is simpler, coatings have the advantages of providing some barrier to water vapor, oxygen, and volatiles, being thus expected to reduce moisture absorption, loss of nutrients and flavor by dehydrated fruits. Alginate is especially interesting as a matrix for probiotic-containing coatings, due to its polyanionic character that may provide a pH-responsive protection of the bacteria in stomach and release in the small intestine (Mei et al., 2014).

The world production of strawberries was around 8.3 million tons in 2018 (FAO, 2018). Strawberries are very popular fruits, due to their peculiar flavor properties. However, they are highly perishable due to tenderness (which makes them extremely susceptible to mechanical damages), high respiration rates and susceptibility to fungal deterioration (Matar et al., 2020), and that is the main reason why strawberries have been frequently commercialized as frozen or dehydrated fruit in order to extend their shelf life.

The objective of this study was to obtain dehydrated strawberry halves containing probiotic *B. coagulans* by two alternative probiotic incorporation methods (i.e. impregnation and coating) and two drying methods (freeze drying and oven drying). The performance of each method combination was comparatively evaluated in terms of physical, chemical, and structural properties of dehydrated strawberries, as well as on their sensory acceptance and capacity to deliver probiotics to the small intestine. This is the first study to compare the performance of impregnation and coating as probiotic incorporation methods, and also the first one to propose *B. coagulans* as a probiotic in dehydrated fruits.

2. Materials and methods

2.1. Preparation of the probiotic strain

Freeze-dried *Bacillus coagulans* BC4 50 MLD spores (lot C235515A) standardized with maltodextrin and containing about 10^{11} cfu g^{-1} were provided by Sacco (Cadorago, Italy). A stock culture was prepared by inoculating 1 g of the freeze-dried culture in 10 mL of tryptone glucose yeast extract (TGY) broth, incubating it in a shaker (37 °C, 150 rpm, 48 h), centrifuging it, then inoculating the biomass into 45 mL of TGY broth, incubating it again (37 °C, 150 rpm, 48 h), centrifuging it, and finally inoculating the biomass into 40 mL of TGY broth added with 10 mL glycerol. The stock culture was stirred in vortex tubes and transferred onto cryogenic tubes for storage at −80 °C.

A 25 mL sample of the frozen stock culture was transferred to 225 mL of TGY medium, incubated for 24 h at 39 °C in an incubator shaker at 200 rpm, and centrifuged (3000 *g*, 15 min). The supernatant was discarded, and the bacterial biomass was inoculated in 225 mL of a spore-forming medium (composed of: Corn Steep Liquor, 5 mL/L; dextrose, 1 g/L; manganese sulfate, 0.056 g/L; calcium carbonate, 0.05 g/L; and ammonium sulfate, 0.5 g/L) at 39 °C, 200 rpm for 48 h. The culture medium was then centrifuged (3000 *g*, 15 min) and washed twice with 40 mL sterile distilled water. The bacterial biomass was then suspended in 40 mL sterile distilled water, in an amount previously calculated for a probiotic concentration of 10 log cfu.mL^{-1}. The viable cell counting consisted of immersing 1 mL samples (in triplicate) into 9 mL of a sterile peptone saline solution (0.85% NaCl, 0.1% peptone), vortex-homogenizing it for 10 s, 6-fold serially diluting in saline solution, and plating (in triplicates) on TGY agar (TGY broth supplemented with 1.5% agar) to determine the viable cell counts (spread plate method). The plates were incubated at 37 °C, and colonies were counted after 48 h.

The spore culture was then stored at −18 °C until use for impregnation suspensions or coating dispersions.

2.2. Processing of probiotic strawberries

The strawberries were purchased from a single supplier in São Carlos, SP, Brazil. They were washed with neutral detergent, rinsed, disinfected with chlorinated water (100 mg/L) for 5 min, rinsed with distilled water, and superficially dried by using sterile gauze. The calyces were then removed, and the strawberries were longitudinally cut into halves.

The probiotic bacteria was included in both an impregnation suspension (without a biopolymer) and a coating dispersion (with alginate). The first one consisted on 500 mL of sterile distilled water containing an amount of the bacterial biomass calculated so as to provide the suspension with a cell count of 8 log cfu.mL^{-1}. The suspension was homogenized with a mechanical stirrer (Ika Eurostar 60 Control, IKA-Werke GmbH, Staufen, Germany) at 650 rpm for 20 min. The coating dispersion consisted of a 1% (w/v) sodium alginate (TICA-algin 400 F, lot 41369, Tic Gums, White Marsh, MD, USA) dispersed in sterile distilled water containing 30 wt% sorbitol (on an alginate basis), and homogenized at 15,000 rpm for 15 min in an Ultra-Turrax T18 homogenizer (IKA-Werke, Staufen, Germany). After homogenization, an amount of the bacterial biomass was added so as to provide the dispersion with a cell count of 8 log cfu.mL^{-1}, and homogenized for 20 min in the Eurostar 60 Control mechanical stirrer at 650 rpm.

The strawberry halves were divided into six groups, each one containing 1.2 kg. Two groups were the controls (not incorporated with probiotics), while two were the impregnation groups, and the other two were the coating groups. The fruit pieces of the impregnation groups were dipped into the impregnation probiotic suspension for 30 min with stirring (60 rpm). The strawberry halves of the coating groups were dipped into the sodium alginate/probiotic dispersion for 1 min, then into a 1% $CaCl_2$ solution (w/v) in sterile distilled water for 1 min, and rinsed in sterile distilled water for 10 s to remove any remaining $CaCl_2$ (not involved in crosslinking with alginate).

From each two groups that received the same probiotic incorporation protocol, one group was pre-frozen in an ultra-freezer at −25 °C for 24 h, then freeze-dried in a Liotop L101 freeze-dryer (Liotop, São Carlos, SP, Brazil) at 41 °C for 8 days. The other group was oven-dried in a Solab SL102 air-circulating oven (Solab, Piracicaba, SP, Brazil) for 48 h at 50 °C.

The six groups/treatments (Fig. 1) are hereafter referred to as: FD (freeze-dried, no probiotic incorporation); OD (oven-dried, no probiotic incorporation), I-FD (impregnated with probiotic and freeze-dried), I-OD (impregnated with probiotic and oven-dried), C-FD (coated with alginate/probiotic dispersion and freeze-dried), and C-OD (coated with alginate/probiotic dispersion and oven-dried). After processing, the strawberry halves from all treatments were packed into zip-lock low density polyethylene bags (0.1 mm in thickness) and stored at a climatic chamber (420-2 TS, Ethik Technology, Vargem Grande Paulista, SP, Brazil) at 25 °C and 50% RH.

2.3. Sensory acceptance

Since the gathering restrictions imposed by the covid-19 pandemic stopped the team from conducting a conventional sensory analysis in a laboratory with individual cabins, a simplified acceptance test was carried out by delivering packages containing six small plastics bags, each containing a sample coded with 3 random digits, along with instructions for the analysis. Fifty-two consumers with ages ranging from 18 to 65 years participated in the test by filling an online form, indicating their degree of overall acceptance of each sample by using a 5-point hedonic scale (from 1 = extremely disliked to 5 = extremely liked). The form included space for comments about what the consumers liked or disliked about each sample. The study was reviewed and approved by the Human Research Ethics Committee of the Centro Universitário Central Paulista (CAAE 18628019.9.0000.5380).

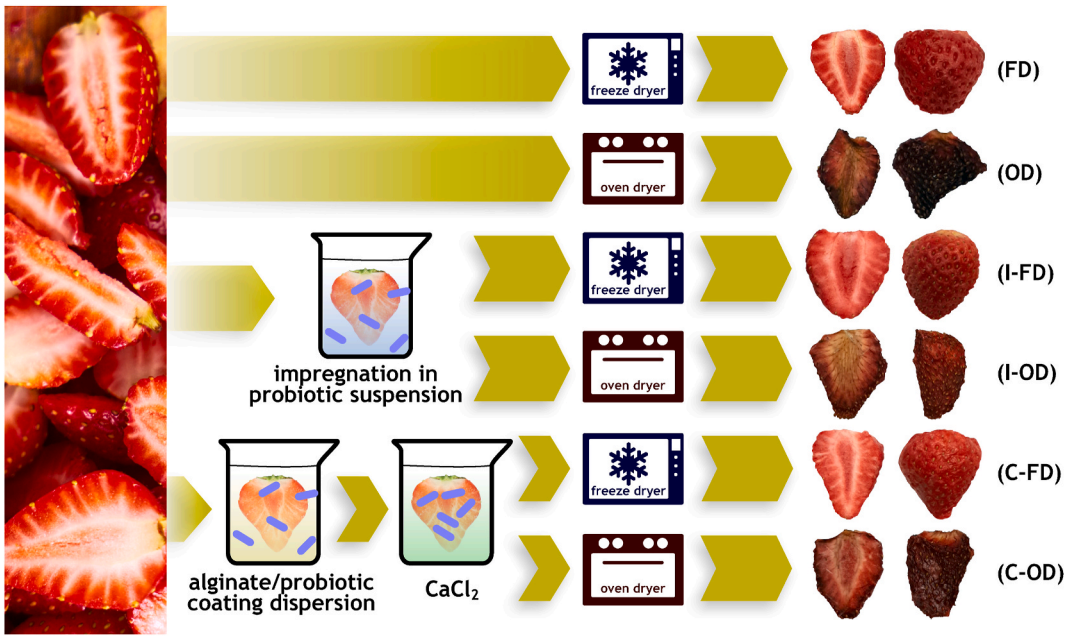


Fig. 1. Treatments on strawberry halves and visual appearance.

2.4. Characterization of probiotic strawberry halves for changes on processing and storage

The following determinations were made before and after dehydration, in order to evaluate the effect of processing (dehydration) on them. Moreover, the determinations were made after 6 months of storage at 25 °C (except for viable cell counting, which was carried out monthly for the 6 months of storage).

2.4.1. Viable cell counting

The changes in viability of the probiotic bacteria along the processing and storage of strawberries were monitored by viable cell counting on samples of all probiotic-containing treatments (I-FD, C-FD, I-OD, and C-OD). Three 2.5 g samples were homogenized into 247.5 mL peptone saline water (0.85% NaCl, 0.1% peptone) in a stomacher for 2 min, then 5-fold serial dilutions (from 10^{-3} to 10^{-7}) were plated (in triplicate) on TGY agar to determine the viable cell counts by the spread plate method. The plates were incubated at 37 °C, and colonies were counted after 48 h. All the viable cell counts were expressed as cfu.g^{-1} (on a dry basis).

2.4.2. Strawberry skin color

The color measurements were made from the strawberry external surface (on the skin), using a Konica Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan) equipped with a C illuminant, using the CIELAB scale. Measurements were taken from five strawberry halves, in triplicate for each one. The total color difference (ΔE*) was calculated according to Eq. (1). ΔE* for processing (ΔE^*_P) was defined as representing the difference between the processed samples (just after dehydration) and fresh strawberries, whereas ΔE* for storage (ΔE^*_S) represented the difference between the end (6 months) and the beginning of storage.

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (1)$$

where ΔL*, Δa*, and Δb* are the differences in L*, a*, and b* average values between processed and fresh strawberries (for processing, ΔE^*_P) or between end and beginning of storage (ΔE^*_S).

2.4.3. Anthocyanins

The anthocyanin contents were determined (in triplicate) by the single pH method based on the 535 nm absorbance measured at pH 2, as previously described (Soquetta, Schmaltz, Wesz Righes, Salvalaggio, & Terra, 2018).

2.4.4. Ascorbic acid

The ascorbic acid determinations were made according to the method proposed by Bresolin and Hubinger (2014, pp. 497–500). Weighed (0.1 g) samples were transferred into 10-mL graduated flasks and made up to 10-mL mark with metaphosphoric acid 3% (w/v), then filtered through a disposable hydrophilic Teflon filter (0.45 μm) and placed in a vial covered with aluminum foil. The samples (30 μL) were injected into the High Performance Liquid Chromatograph (HPLC) Varian with dual pumps (Pro Star 210) and an UV–Vis detector (Pro Star 325) adjusted for 245 nm. The mobile phase was phosphate buffer pH 2.5, with a flow rate of 1.0 mL/min. Separation was performed on an Agilent C18 (2.5 × 25 mm, 5 μm) column. The L-ascorbic acid (purity ≥ 99.0%) used as a standard was obtained from Sigma Life Science (V000200).

2.4.5. Firmness

Firmness (expressed as N) was measured using a texturometer (Stable Micro System, model TA-XT.Plus, Surrey, UK) with a 4 mm plunger at a shearing speed of 1 mm s^{-1} to a depth of 5 mm. Five measurements were taken for each treatment (one strawberry half per measurement).

2.4.6. Scanning electron micrography (SEM)

Sections (10 mm^2, 1 mm in thickness) were dissected from the strawberry surfaces for scanning electron microscopy (SEM). The specimens were fixed to aluminum stubs using conductive carbon tape and sputter-coated with a 10 nm-thick gold layer by using the ACE600 Sputter Coater (Leica Microsystems, Wetzlar, Germany). The fractured surfaces were obtained by submerging strawberry halves in liquid nitrogen for 5 min and fracturing with tweezers. The specimens were mounted onto aluminum stubs with the fractured surface facing upward, using conductive carbon tape, then sputter-coated with a 10 nm-thick gold layer. The specimens were observed under a JSM 6510 (Jeol, Tokyo, Japan) microscope at 10 kV, the surfaces and fractures at 5000 × and 100× magnifications respectively.

2.5. Viability of the probiotic strain on simulator of human microbial ecosystem (SHIME®)

SHIME® is a dynamic model composed of five double-jacketed vessels representing stomach (vessel 1), small intestine (vessel 2), as well as ascending, transverse and descending colon (vessels 3–5 respectively) of the human gastrointestinal tract. In this study, only the vessels 1 (stomach) and 2 (small intestine) were used. The system is connected with a software that controls the pH, residence time, and temperature of each vessel, as previously described (Molly, Woestyne, Smet, & Verstraete, 1994).

The feeding medium was composed of corn starch (3 g/L), pectin (2 g/L), mucin (4 g/L), xylan (1 g/L), peptone (1 g/L), arabinogalactan (1 g/L), glucose (0.4 g/L), yeast extract (3 g/L), and cystein (0.5 g/L) in distilled water. Strawberry samples (6 g) from the probiotic-containing treatments were diluted to 10^2 in this medium, homogenized in a stomacher at 230 rpm for 2 min, and transferred to the vessel 1, where it was kept for 2 h at 37 °C at a pH of 2.5–2.9. The content of vessel 1 was then transferred to the vessel 2 and incubated for 4 h at 37 °C. The small intestine conditions were simulated by adding artificial pancreatic juice (12.5 g/L of $NaHCO_3$, 6 g/L of Oxgall, and 0.9 g/L of pancreatin) at a rate of 4 mL/min for 15 min. The homogeneity of the samples in each vessel was maintained by using a magnetic stirrer.

At the end of the process, samples of strawberry fragments (1 g) and the small intestine fluid (1 mL) were collected (in triplicate) and suspended into 9 mL of a sterile peptone saline solution (0.85% NaCl, 0.1% peptone), then submitted to the viable cell counting as previously described.

2.6. Data processing and statistical analyses

The changes on processing were evaluated by comparing the properties of the processed strawberry halves with those of the fresh strawberries (on a dry basis). The changes on storage, on the other hand, were assessed by comparing the properties of the strawberry halves at the end of the storage time with those just after processing (storage time 0).

The data were analyzed using the general linear model (two-way Anova) of Minitab® statistical software v. 19 (Minitab Inc., State

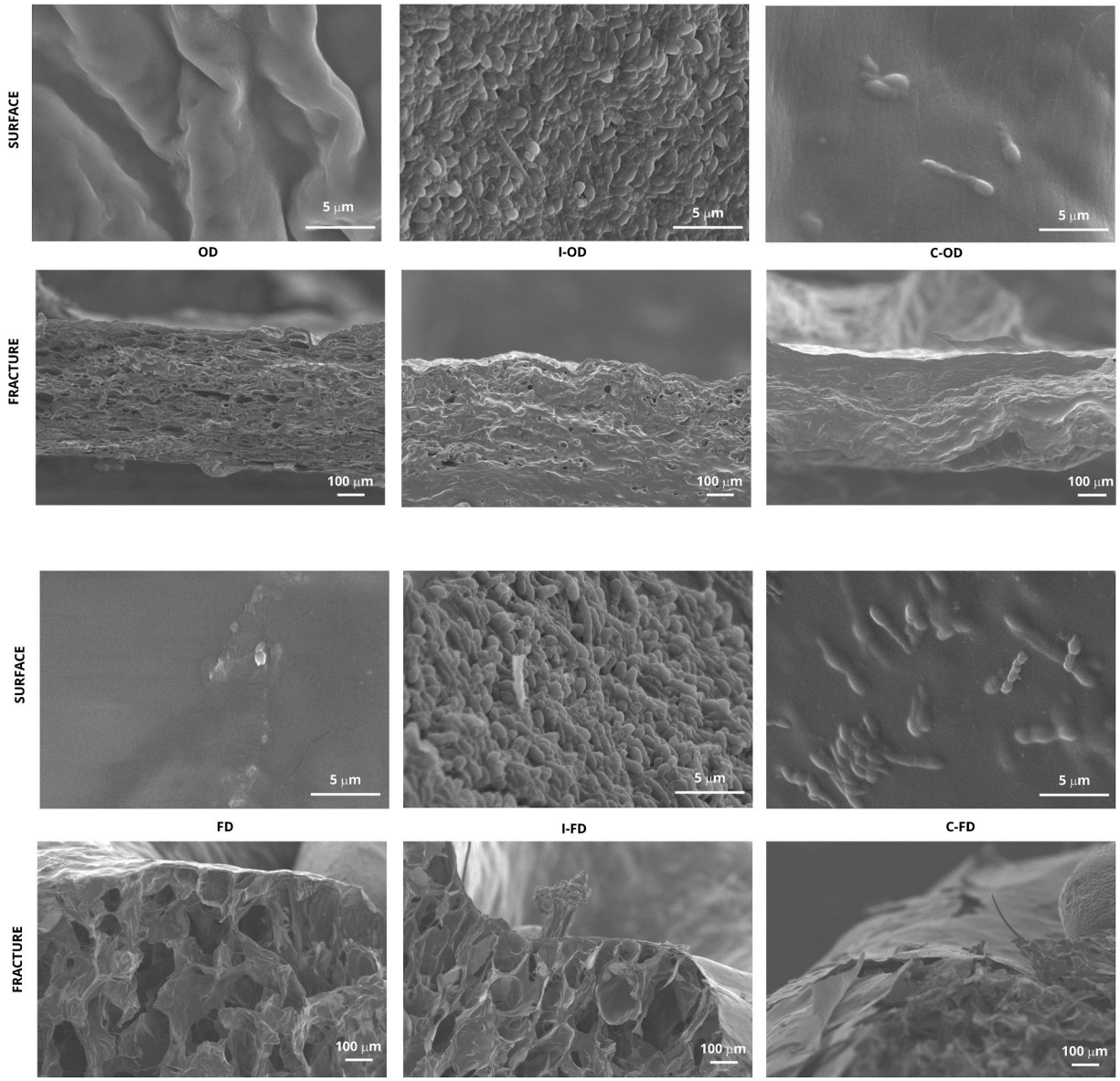


Fig. 2. Scanning electron micrographs of surfaces and fractures of strawberry halves submitted to the treatments. OD: oven dried (no probiotic); C-OD: alginate-probiotic-coated and oven dried; I-OD: impregnated with probiotic suspension and oven dried; FD: freeze dried (no probiotic); C-FD: alginate-probiotic-coated and freeze dried; I-FD: impregnated with probiotic suspension and freeze dried.

College, PA, USA). When significant differences were found ($p < 0.05$) for a categorical factor (type of processing or form of probiotic incorporation), comparisons were made (Tukey's multiple comparisons test for comparison of three groups, or t-tests for comparison of two groups, $p < 0.05$). When a continuous variable was involved (time of storage), regression analysis and Anova were performed in order to asses the significance of the factors.

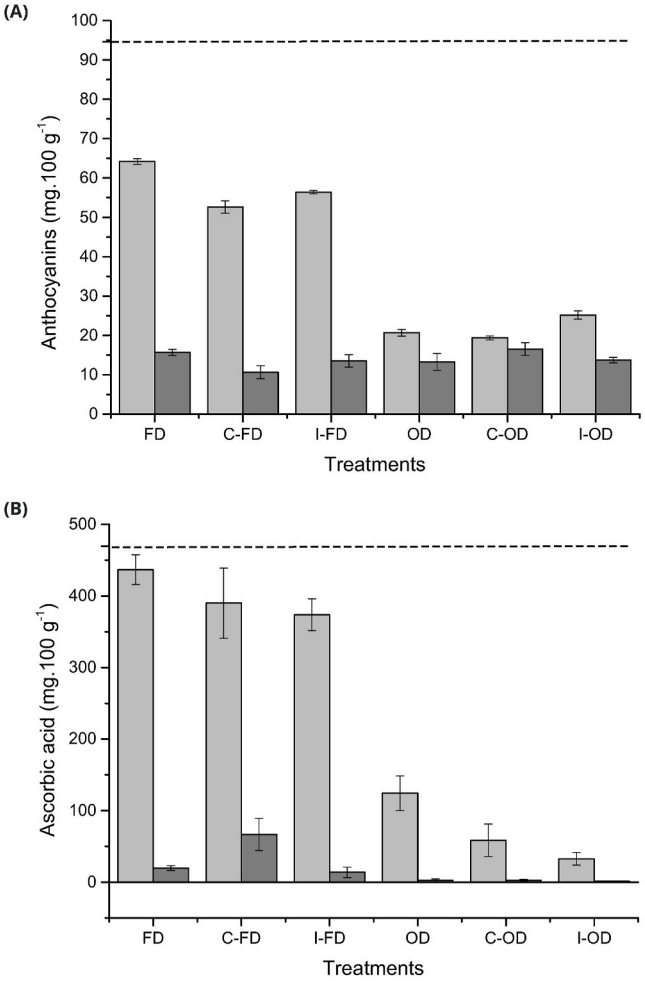
3. Results and discussion

3.1. Microstructure of the dehydrated strawberry halves

The dehydration methods produced quite different microstructures on both surfaces and fractures of strawberry halves (Fig. 2). Whereas the oven dried samples exhibited rougher surfaces (visible especially at the surface of OD) and collapse of the fruit structure, freeze dried strawberries presented large pores, demonstrating the preservation of cell structures, corroborating previous results with banana and mango (Zotarelli, Porciuncula, & Laurindo, 2012) Those differences are consequences of the damages to the fruit tissues by oven drying, which involves destruction of the porous structure due to capillary forces, whereas freeze drying avoids the liquid/vapor interface and involves sublimation at the solid/vapor interface, eliminating capillary forces (Wang, Fang, Ye, Zhang, & Feng, 2020). The surfaces of impregnated samples were covered by bacteria, while the coated ones (especially the C-FD) exhibited the contours of bacteria embedded in the alginate matrix as clusters rather than a uniform bacterial layer, similarly to lactic acid bacteria in whey protein (Pereira et al., 2016) and starch/carboxymethylcellulose films (Li et al., 2020).

3.2. Chemical and physical changes on processing and storage

One of the problems of thermal processing methods is the thermal degradation of heat-sensitive compounds, including nutrients (such as ascorbic acid) and pigments (such as anthocyanins), thus reducing sensory, nutritional, and antioxidant values of foods. Indeed, whereas the mean losses of anthocyanins and ascorbic acid on oven drying of strawberry halves were about 77% and 85% respectively, those losses were around 39% and 15% for the freeze dried samples (Fig. 3), corroborating previous studies reporting much higher retention of heat-sensitive compounds on freeze drying than oven drying (Samoticha, Wojdyło, & Lech, 2016). The method of probiotic incorporation also affected the retention of both anthocyanins and ascorbic acid on processing. Surprisingly, coating or impregnation with probiotics increased the mean losses of those compounds on processing, which may be ascribed to a leaching effect by the probiotic suspension or coating dispersion, since both anthocyanins and ascorbic acid are water-soluble. An additional factor that might have contributing to this effect is dilution by the probiotic bacteria and/or coating materials, since the losses were calculated on a dry basis of fresh strawberries (without coating or



Losses on processing

Factors	Groups	Means (%)	p
Processing	Oven drying	77.05	<0.01
	Freeze drying	39.00	
Probiotic incorporation	No probiotic	55.18 B	0.02
	Coating	61.98 A	
	Impregnation	56.91 AB	

Losses on storage

Factors	Groups	Means (%)	p
Processing	Oven drying	31.99	<0.01
	Freeze drying	77.14	
Probiotic incorporation	No probiotic	55.69	0.09
	Coating	47.28	
	Impregnation	60.74	

Losses on processing

Factors	Groups	Means (%)	p
Processing	Oven drying	84.70	<0.01
	Freeze drying	14.76	
Probiotic incorporation	No probiotic	40.24 B	<0.01
	Coating	52.23 A	
	Impregnation	56.73 A	

Losses on storage

Factors	Groups	Means (%)	p
Processing	Oven drying	96.70	0.02
	Freeze drying	91.57	
Probiotic incorporation	No probiotic	96.75 A	0.01
	Coating	89.25 B	
	Impregnation	96.40 A	

Fig. 3. Anthocyanin (A) and ascorbic acid (B) contents of strawberry halves (on a dry basis) and statistical analyses of losses on processing and storage. FD: freeze dried (no probiotic); C-FD: alginate-probiotic-coated and freeze dried; I-FD: impregnated with probiotic suspension and freeze dried; OD: oven dried (no probiotic); C-OD: alginate-probiotic-coated and oven dried; I-OD: impregnated with probiotic suspension and oven dried. Dotted line: before processing. Light gray bars: after processing. Dark gray bars: after storage (6 months).

probiotics).

The losses on storage, on the other hand, presented unusual variations. Whereas freeze dried strawberry halves exhibited much lower anthocyanin retention when compared to those of oven dried samples, their ascorbic acid retention was slightly (but significantly) higher. On the one hand, their lower anthocyanin retention may be ascribed to their higher surface area-to-volume ratio due to the high porosity of the fruit pieces, promoting an increased O_2 exposure, leading to higher anthocyanin oxidation (Sarkis, Jaeschke, Tessaro, & Marczak, 2013). On the other hand, it is hypothesized that their higher ascorbic acid retention on storage is partially explained by the protecting effect of antioxidant compounds that may have been more retained on the freeze dried strawberry tissues than on oven dried ones (Dorta, Lobo, & González, 2012). The method of probiotic incorporation influenced the ascorbic acid retention on storage, the coating method having improved the retention, probably by decreasing oxidation promotedlb by the O_2 exposure (Sarkis et al., 2013), since alginate, being hydrophilic, has a good barrier against O_2.

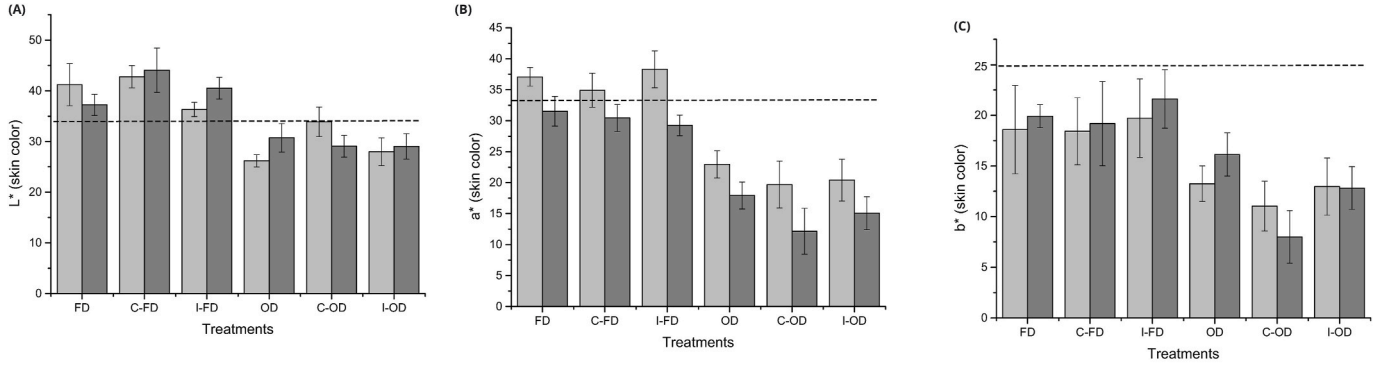
After processing, the freeze dried strawberry halves tended to be brighter (higher L*, Fig. 4A) due to increased light scattering by the pores formed on sublimation (Ceballos, Giraldo, & Orrego, 2012), with increased redness (a*, Fig. 4B) and decreased yellowness (b*, Fig. 4C) due to increased anthocyanin concentration by water removal. The oven dried samples were darker, with decreased a*, due to non-enzymatic browning related to Maillard reactions, acid-catalyzed sugar degradation and ascorbic acid degradation, as well as anthocyanin degradation (Buvé et al., 2018). The total color changes (ΔE*) were higher on oven drying than freeze drying, and not affected by the method of probiotic incorporation. The main color change on storage of all samples was the decreased a* (Fig. 4B), related to anthocyanin loss (Fig. 3), but the ΔE* on storage was not significantly affected by the processing method or probiotic incorporation.

The firmness of strawberry halves (Fig. 5) was noticeably affected by the processing method, the oven dried samples being much firmer, which is ascribed to the shrinkage of the solid matrix resulting from the rapid water removal causing microstructural stresses (Pei et al., 2014; Zotarelli et al., 2012), whereas freeze drying results in a porous and less dense texture, with the cell structures mostly intact (An et al., 2016). In contrast, the firmness of the freeze dried samples was more affected by storage than those of oven dried ones (although the final firmnesses of freeze dried strawberries have still been a fraction of those of the oven dried strawberries), due to a partial collapse of the porous structure. The method of probiotic incorporation did not affect the firmness changes on processing, but the impregnation method resulted in a lower increase in firmness on storage when compared to the other probiotic incorporation methods, which may be ascribed to some structuring role of the impregnated bacteria, imparting some robustness to the matrix (Santivarangkna, Aschenbrenner, Kulozik, & Foerst, 2011).

3.3. Sensory acceptance

The acceptance of strawberry halves was significantly affected by the processing method (Fig. 6), the freeze-dried samples being better accepted than the oven-dried ones, since freeze-drying is a technique that minimizes the thermal damages promoted by oven drying on flavor and color compounds as well as in physical cell structure (An et al., 2016; Torres, Díaz-Maroto, Hermosín-Gutiérrez, & Pérez-Coello, 2010). Indeed, the appearance of freeze dried samples was much more similar than that of oven dried ones (Fig. 1), since oven drying results in profound changes in color (causing browning reactions as well as anthocyanin degradation, as previously mentioned) as well as shrinkage. The shrinkage of foods upon oven drying is dependent on the volume of water removed, causing contraction stresses that make the cell structures to collapse, whereas minimal shrinkage occurs in freeze dried products, since the water is removed by sublimation, keeping the cell structures intact (Baeghbali, Ngadi, & Niakousari, 2020; Fauster, Giancaterino, Pittia, & Jaeger, 2020).

Negative comments on the appearance, texture, and flavor of oven-dried samples were frequent (Fig. 6), which are ascribed to the thermal damages to strawberry color (as observed from 4), the shrinkage effects from water evaporation causing hardness (Fig. 5) and poor appearance, and thermal degradation of flavor compounds. The only negative comment on freeze dried samples was the "styrofoam-like" texture, which may be ascribed to the porous, honeycomb-like cellular structure resulting from freeze drying. Such an effect could be avoided by rehydration of strawberry halves before consumption, since freeze dried samples have high rehydration capacity due to their maintained cell structure (Fauster et al., 2020). Positive comments on flavor, appearance, and texture were frequent for freeze dried samples, which are derived from the very lack of the thermal-induced adverse effects resulting from oven drying. The probiotic incorporation method did not affect the acceptability of the products, indicating that the presence of



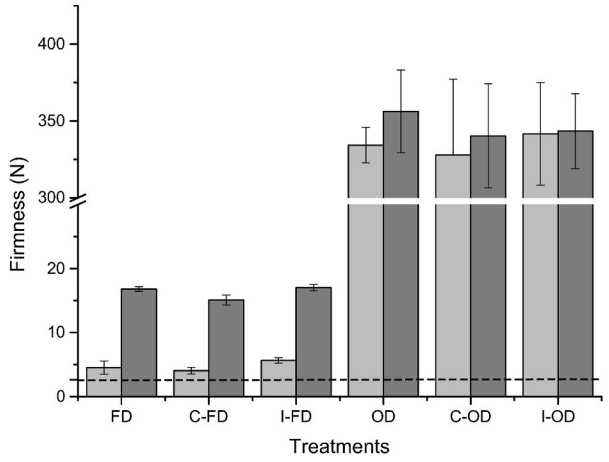
ΔE* on processing

Factors	Groups	Means	p
Processing	Oven drying	18.77	<0.01
	Freeze drying	10.62	
Probiotic incorporation	No probiotic	14.51	0.32
	Coating	15.78	
	Impregnation	13.80	

ΔE* on storage

Factors	Groups	Means	p
Processing	Oven drying	8.10	0.07
	Freeze drying	8.37	
Probiotic incorporation	No probiotic	7.63	0.38
	Coating	8.70	
	Impregnation	8.37	

Fig. 4. Total skin color differences of strawberries on processing and storage. Dotted line: before processing. Light gray bars: after processing. Dark gray bars: after storage (6 months).



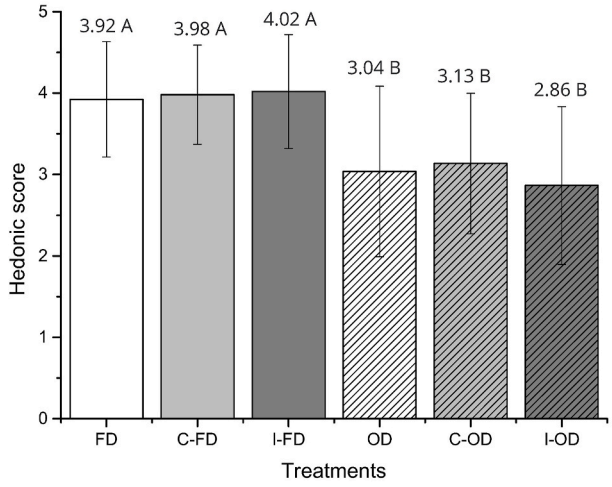
Changes on processing

Factors	Groups	Means (times)	p
Processing	Oven drying	126.1	<0.01
	Freeze drying	0.8010	
Probiotic incorporation	No probiotic	63.37	0.78
	Coating	62.07	
	Impregnation	64.96	

Changes on storage

Factors	Groups	Means (%)	p
Processing	Oven drying	3.638	<0.01
	Freeze drying	248.4	
Probiotic incorporation	No probiotic	139.0 A	<0.01
	Coating	138.1 A	
	Impregnation	100.9 B	

Fig. 5. Firmness changes on processing and storage. Dotted line: before processing. Light gray bars: after processing. Dark gray bars: after storage (6 months).



Frequent comments	Frequency (%)					
	FD	C-FD	I-FD	OD	C-OD	I-OD
NEGATIVE						
Bad/poor appearance	-	-	-	5.8	13.5	5.8
Too tough	3.8	-	-	17.3	25	21.2
Styrofoam-like texture	7.7	7.7	5.8	-	-	-
Lacking strawberry flavor	-	-	-	15.4	7.7	9.6
POSITIVE						
Good/nice appearance	7.7	11.5	7.7	-	-	-
Good/nice texture	3.8	7.7	11.5	-	-	-
Typical strawberry flavor	15.4	11.5	11.5	-	-	-

Factors	Groups	Means	p
Processing	Oven drying	3.01	<0.01
	Freeze drying	3.97	
Probiotic incorporation	No probiotic	3.48	0.59
	Coating	3.56	
	Impregnation	3.44	

Fig. 6. Sensory acceptance of strawberry halves from the different treatments and frequent comments by evaluators. FD: freeze dried (no probiotic); C-FD: alginate-probiotic-coated and freeze dried; I-FD: impregnated with probiotic suspension and freeze dried; OD: oven dried (no probiotic); C-OD: alginate-probiotic-coated and oven dried; I-OD: impregnated with probiotic suspension and oven dried.

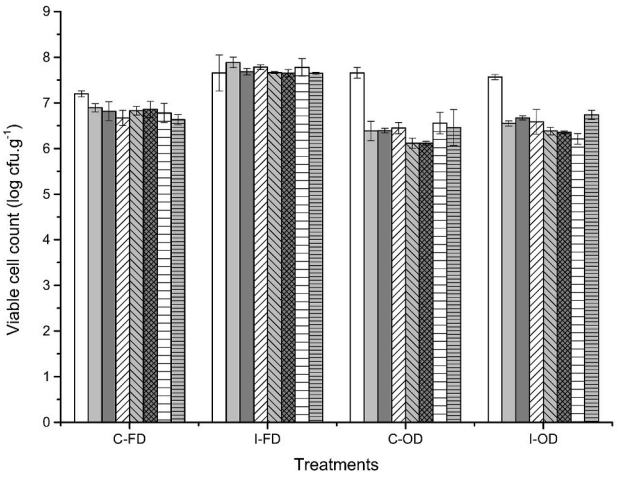
probiotic bacteria and/or alginate coatings were either not perceived by the consumers or not influential to the overall acceptance of the samples. While the presence of the bacteria was indeed not expected to affect acceptance in any way, the existence of polysaccharide coatings on the surface of fruit samples has been previously reported to affect acceptance of fruit samples, either negatively as in whole papaya (Batista et al., 2020) and fresh-cut pineapple (Prakash, Baskaran, & Vadivel, 2020) or positively as in strawberries (Tahir et al., 2018).

3.4. Changes in probiotic viability on processing and storage

Even though *B. coagulans* is spore-forming, its viability (Fig. 7) was more affected by the thermal drying method (oven drying) than freeze drying, since even spores are affected (although in a lower extent than vegetative cells) by higher temperatures (Luu-Thi, Khadka, & Michiels, 2014; Somavat, Mohamed, & Sastry, 2013). Moreover, the impregnation method resulted in higher viability retention than coating. Although the coating approach involves a polysaccharide matrix to protect the probiotic cells (Espitia, Batista, Azeredo, & Otoni, 2016), the higher effectiveness of the impregnation technique to protect the probiotic may be ascribed to the bacteria penetrating more deeply into the strawberry tissues, and being thus protected by the fruit matrix itself (Betoret et al., 2019). The bacteria viability on storage was not significantly affected by storage time, but only by the processing and probiotic incorporation methods, as direct consequences from the previous differences on the processing step. The I-FD treatment was the one that kept the highest probiotic cell counts after processing and, accordingly, throughout storage (near 8 log cfu.g^{-1}).

3.5. Probiotic viability on SHIME®

After the passage through SHIME®, the probiotic cell counts (Fig. 8) made it clear that the probiotics survived well the passage though the stomach, which is not surprising, given the spore-forming ability of *B. coagulans*, making it more resistant to the low pH of the stomach due to the hard protein coat of the spores ((Ahire, Neelamraju, & Madempudi, 2020; Setlow, Atluri, Kitchel, Koziol-Dube, & Setlow, 2006), corroborating previous findings (Marcial-Coba et al., 2019; Shinde et al., 2019). It was recently observed that *B. coagulans* survived under fed and fasted gastrointestinal conditions, and the highest spore germination



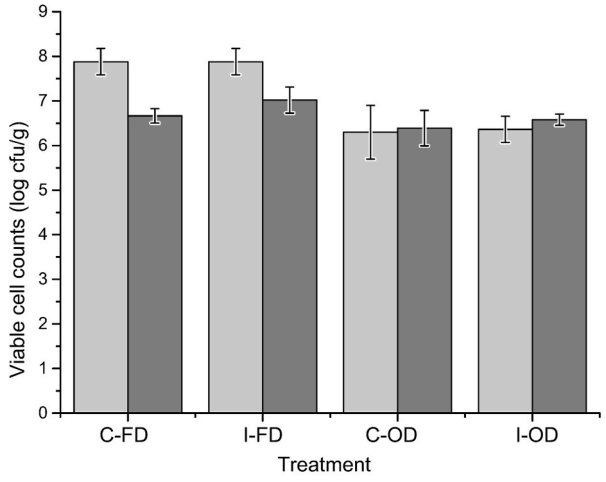
ANOVA - Losses on processing

Factors	Groups	Means (log cfu.g^{-1})	p
Processing	Oven drying	1.14	<0.01
	Freeze drying	0.198	
Probiotic incorporation	Coating	0.787	0.01
	Impregnation	0.556	

Regression analysis/ANOVA - Viability on storage

Source	F-value	p
Regression	93.26	<0.01
Storage time	0.51	0.47
Processing	204.36	<0.01
Probiotic incorporation	82.25	<0.01

Fig. 7. Changes in viability of *B. coagulans* on processing and storage of strawberry halves. White bars: before drying. Light gray bars: after drying. Dark gray bars: after 30 days of storage. White bars, diagonal hatches: after 60 days of storage. Light gray bars, diagonal hatches: after 90 days of storage. Dark gray bars, diagonal cross hatches: after 120 days of storage. White bars, horizontal hatches: after 150 days of storage. Light gray bars, horizontal hatches: after 180 days of storage.



ANOVA - Cell counts on the intestine fluid

Factors	Groups	Means	p
Processing	Oven drying	6.48	0.04
	Freeze drying	6.84	
Probiotic incorporation	Coating	6.53	0.10
	Impregnation	6.80	

Fig. 8. Probiotic viable cell counts on strawberry fragments and the small intestine fluid after the passage through the simulator of human microbial ecosystem (SHIME®). Light gray bars: strawberries. Dark gray bars: small intestine fluid.

was detected in small intestine in an *in vitro* simulated model of the gastrointestinal tract (Ahire, Neelamraju, & Madempudi, 2020).

Although the strawberry fragments kept high cell counts even after passing through the small intestine, the bacteria have also been widely released to the small intestine fluid. The freeze dried samples released significantly more probiotics than the oven dried ones (which is probably ascribed to the bacteria transport being facilitated by a more porous structure), but there was no significant differences between the probiotic incorporation methods. Anyway, the strawberry halves of all treatments were able to release probiotics in counts higher than 6 log cfu.g^{-1} to the small intestine, indicating that all proposed treatments were able to result in products that could be claimed as probiotic snacks. On the other hand, when all results are considered (especially the ones related to retention of bioactives – namely, ascorbic acid and anthocyanins – and sensory acceptance), the treatment based on freeze drying probiotic-impregnated strawberry halves showed the best overall performance to produce a well accepted functional snack.

4. Conclusions

There were noticeable differences between drying methods in terms of the resulting properties of strawberry halves, freeze drying having provided the fruit pieces with a better preservation of their properties on processing, including higher retention of bioactives, shape, color, and firmness, which is ascribed to the low temperatures (avoiding adverse thermal changes) and the preservation of cell structures thanks to sublimation eliminating capillary forces. Moreover, freeze drying kept a higher probiotic viability when compared to oven drying, resulting in higher viable cell release to the small intestine, which was the ultimate purpose of the presence of probiotics in the products. Additionally, the freeze dried samples presented better sensory acceptance, which was ascribed to the high ability of the technique to retain flavor and color compounds as well as to avoid shrinkage effects. The *B. coagulans* BC4 in the product was able to keep its viability unchanged throughout storage, and was also resistant to the passage through stomach and small intestine, which is probably due to its spore-forming ability, and consequent high resistance to temperature, pH, and mechanical stresses. The combination of impregnation and freeze drying was the one that resulted in the highest probiotic viability through storage (neat 8 log cfu.g^{-1} along 6 months), making the product promising as a convenient, stable, and well-accepted functional snack.

CRediT authorship contribution statement

Aline Soares Oliveira: Investigation, Methodology. **Carolina Madazio Niro:** Investigation, Methodology. **Joana Dias Bresolin:** Methodology, Supervision, Writing – review & editing. **Viviane Faria Soares:** Methodology, Writing – review & editing. **Marcos David Ferreira:** Conceptualization, Resources, Writing – review & editing. **Katia Sivieri:** Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **Henriette M.C. Azeredo:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111105.

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